Effects of organic and waste-derived fertilizers on yield, nitrogen and glucosinolate contents, and sensory quality of broccoli (*Brassica oleracea* L. var. *italica*)

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ABSTRACT

Organic vegetable production attempts to pursue multiple goals concerning influence on environment, production resources and human health. In areas with limited availability of animal manure, there is a need for considering various off-farm nutrient resources for such production. Different organic and waste-derived fertilizer materials were used for broccoli production at two latitudes (58° and 67°) in Norway during two years. The fertilizer materials were applied at two rates of total N (80 kg ha^{-1} and 170 kg ha^{-1}) and compared with mineral fertilizer (170 kg ha^{-1}) and no fertilizer. Broccoli yield was strongly influenced by fertilizer materials (algae meal<unfertilized control<sheep manure<extruded shrimp shell<anaerobically digested food waste<mineral fertilizer). Yield, but not glucosinolate contents, was linearly correlated with estimated potentially plant-available N. However, extruded shrimp shell and mineral NPK fertilizer gave higher glucosinolate contents than sheep manure and no fertilizer. Sensory attributes were less affected by fertilizer material and plant-available N.

Keywords: glucosinolates, sustainability, *Brassica oleracea*, broccoli, sensory attributes, nitrogen mineralization, yield, organic farming, organic fertilizer.

INTRODUCTION

Organic agricultural production is increasing in Europe [1]. Important reasons are consumers' growing interest in food safety, environmental impact and sustainability of production systems as well as a preconceived notion about a superior quality of organic products with respect to nutrients, compounds with health-promoting properties and taste characteristics [2-6]. Ethical concerns and decrease in consumers' trust in food quality also seem to be among the driving forces [7, 8]. Still, price as influenced by efficiency in the production and distribution chain, including marketable crop yield per unit area, is an important determinant of the consumers' choice [9].

The ban on mineral fertilizers is one of the key characteristics of organic cropping systems. This particularly influences nitrogen (N) availability, which is the single factor that most often limits crop yield [10]. The N availability during the growth of vegetables also influences several quality parameters through nitrogen's functions as building blocks in plant tissues and in metabolic and physiological processes, including synthesis of vitamins and secondary metabolites. Overall, as compared to conventional produce, organic vegetables and fruits tend to have higher contents of defense-related secondary metabolites, which comprise many of the known and supposedly health-promoting compounds in these foods [11]. Previous studies suggest that this difference may be related to N availability in cropping systems [12].

Organic farming systems mainly depend on N_2 fixation in leguminous plants and green manure crops. On stockless farms in areas with few animals, the possibilities are limited locally for utilizing the legumes needed in crop rotations and for recycling nutrients as animal manure. The resulting high cost of N on such farms, therefore, tends to limit the proportion of legumes in the crop rotation. Hence, there is a need for considering various off-farm N sources derived from organic materials [13], particularly for farms producing organic crops with large N demand, such as cruciferous vegetables. Organic waste materials originating from food or seafood production are potentially relevant nutrient sources. Turning such wastes into a production resource by establishing closed nutrient cycles would contribute to sustainable management of both environment and production.

The N fertilizer effect of such resources on crop growth depends on the amount and timing of inorganic N availability in relation to crop demand [14]. The N supply from a specific fertilization source can be described as a function of amount of total N applied, percentage inorganic N at application, decomposition rate of the organic fraction, and carbon-to-nitrogen (C:N) ratio of the fractions available to decomposers. The crop N demand typically follows a sigmoidal pattern and is defined as the N uptake over a period which allows the maximum production of dry matter [15]. An important indicator of N demand is the critical plant N concentration (PNCc), which is the lowest level of N allowing optimum growth [14, 16, 17].

Cruciferous vegetables are important dietary sources of several minerals, vitamins and other health-related components [6, 18, 19]. Especially broccoli (*Brassica oleracea* L. var. *italica*) is considered an important commercial and dietary vegetable, representing a good source of glucosinolates (GLS), phenolic compounds, vitamin C and carotenoids [6, 20, 21]. Consumption of cruciferous vegetables is associated with a reduced risk of certain types of cancer and cardiovascular diseases [18-21], and this has been related to its content of GLS and their degradation products. There is also a general belief among consumers that broccoli is a healthy food [9].

Despite this focus on chemical composition, little is known about effects of different fertilizers on sensory quality and content of GLS. Staley [22] found a higher content of GLS in cabbage fertilized with chicken manure and green manure compared to mineral fertilizer. In a study based on commercial broccoli purchased at monthly intervals during one year, higher levels of glucobrassicin were found in organic broccoli compared to conventional [23]. In addition to N availability, other qualities such as supply of sulfur (S) [24], nitrogen-to-sulfur (N:S) ratio [25] as well as chitin [26] may influence GLS biosynthesis. Sensory attributes of vegetables grown organically and conventionally show inconsistent results as well [27, 28], and no assessment concerning taste of broccoli related to fertilizer materials is known.

The aim of the present study was to investigate effects of potential organic fertilizers on yield, N and GLS contents, and sensory attributes of broccoli grown at two latitudes with different climate. Algae meal (AM), extruded shrimp shell (SS), sheep manure (SM) and anaerobically digested food waste (AD) were applied at two levels of total N (80 kg ha^{-1} and 170 kg ha^{-1}), and compared with no fertilizer (NF) and mineral fertilizer (MF). Particular attention was paid to possible relationships between estimated N mineralization potential of the fertilizers and parameters of yield and crop quality.

MATERIALS AND METHODS

Site description, soil properties and weather data. The experimental fields were located at the Norwegian Institute for Agricultural and Environmental Research, Division Bodø

(Northern Norway, 67°28’N, 14°45’E) and Division Grimstad (Southern Norway, 58°34’N, 8°52’E) during the growing season of 2009 and 2010. The field in Bodø had been organically managed as cattle pasture for more than 25 years, while the field in Grimstad had been used for organic grass seed production (*Phleum pratense* L.) for three years. The field in Bodø was a sandy orthic humo-ferric podzol [29], while the field in Grimstad was a gleyed sombric brunisol [30] with a southwest-facing slope of 2-4% and 2-6%, respectively.

The year prior to the experiments, fields were ploughed (20–30 cm depth) in late July and harrowed (5–10 cm depth) twice (early August and late September) to reduce weeds. Ryegrass (*Lolium multiflorum* var. Westerwoldicum) was sown in plots prior to the experimental years. Soil samples (0–30 cm depth) were randomly taken from each replicate at both locations with a soil auger (6–10 soil cores per sample) in spring. Meteorological data during the experimental period were available on hourly basis from climate stations nearby the research sites (Table 1).

Design and management of the field experiments. Seeds of broccoli *(Brassica oleracea* L. var. *italica* cv. Marathon*)* were sown in plugtrays with 63 ml plant^{-1} of organic peat-based compost (Norsk økotorv, Norgro AS, Ridaby, Norway) supplemented with 3 g l^{-1} of organic chicken manure (Marihøne, Norsk naturgjødsel AS, Voll, Norway). A multi-factorial field experiment, with the fertilizer materials as independent variables, was established as part of a yearly crop rotation (broccoli, potatoes, lettuce). Fertilizer materials were algae meal (AM) (Bioalg regular®, Nordtang AS, Vestbygd Norway), extruded shrimp shell (SS) («Rekeskall Ottar», Produsentorganisasjonen Ottar, Finnsnes, Norway), sheep manure (SM) (Non-commercial product, Organic farm, Tjøtta, Norway) and anaerobically digested food waste

(AD) (Biotek AS, Porsgrunn, Norway) supplied at two levels of N (80 kg N ha^{-1} and 170 kg N ha^{-1}), broadcast by hand, and incorporated to the soil by a rotary harrow. No fertilizer (NF) and 170 kg N ha^{-1} of mineral fertilizer (MF) given by a combination of NPK 12-4-18 and calcium nitrate fertilizers (Kalksalpeter) (59% of N from NPK) both obtained from Yara (Oslo, Norway) were used as control plots. The first year the total amount of organic fertilizers and 50% of the MF were added before planting, whereas the remaining MF was top-dressed twice (25% after 4 and 25% after 6 weeks). The second year, all fertilizers, except AM, were applied the same way as MF (the change was based on first year results, which suggested nutrient runoff). Due to low level of potassium (K) in SS, potassium sulfate (Kaliumsulfat, Kali, Felleskjøpet, Norway) were supplied in SS plots in a level corresponding to the K level given by the other fertilizer materials (fertilizer rate equal to a N:K ratio 1:1). The experimental fields were arranged as a randomized block design with three large plots (30 m $\times$ 5.6 m and 30 m $\times$ 6.4 m in Bodø and Grimstad, respectively), each of which were divided into 10 sub plots (6 $\times$ 2.8 m and 6 $\times$ 3.2 m in Bodø and Grimstad, respectively). Six week old seedlings were transplanted the first week of June in rows of 18 plants and four rows per fertilizer plot. The distance between plants in the row was 33 cm, and the distance between rows was 70 cm and 80 cm in Bodø and Grimstad, respectively. The experimental fields were covered by floating row cover as insect net (Novagryl floating row cover, 22g m^{-1}, pr. no. 255094, Vekstmiljø AS, Sandnes, Norway).

Nutritional status of soil and organic fertilizers. The soil samples and organic fertilizers were analyzed by Eurofins (Eurofins Food & Agro Testing Norway AS, Moss, Norway). Samples of soil and organic fertilizer materials were dried at 40°C, strained through a 2 mm sieve, and ground in a mortar before analysis. Total carbon (TC) in soil samples for Grimstad and total N (TN) in soil samples from both locations, were determined according to AJ31, a modified version of NS-EN 13137:2001. TC data for Bodø present in Table 2 was analyzed by Haraldsen et al [29]. For the organic fertilizer materials, total organic carbon (TOC) was determined according to NS-EN 1484 and AJ31, whereas total Kjeldahl N (TKN) was analyzed according to NS-EN 13654-1 and Tecator ASN 3503/300.

NO_3^--N and NH_4^+-N was extracted using 2M KCl, while for determination of phosphor (P), potassium (K) and sulfur (S) samples were digested in 7M HNO_3. NO_3^--N, NH_4^+-N, P, K and S were determined according to NS-EN ISO 11885. Soil properties for the field locations and nutritional status of the organic fertilizers are given in Table 2 and Table 3, respectively.

Sampling and sample preparation. Broccoli heads were harvested at maturity of individual plants as defined by developmental stage of flower buds (closed bud diameter of 1–1.5 mm, before elongation of bud stem) and by the density of heads (shift from compact and hard to slightly softer when finger pinching the top of the heads). Broccoli heads that failed to reach normal and uniform bud maturity were harvested when primary buds in the florets started stem elongation and extended 2–3 mm above undeveloped flower buds (some single buds fulfilled development). Weight of individual broccoli heads was measured, and total yield was calculated as the weight of all broccoli heads harvested in plots divided by harvested area (14.8 and 16.9 m^2 for Bodø and Grimstad, respectively). Total number of harvested broccoli heads per plant and fraction of small heads (diameter <6 cm) were also recorded (according to NS 2823:1999).

For sensory and chemical analyses, ten broccoli heads were divided into florets of 10–30 g with 2 cm floret stem, and 50 florets per treatment were randomly selected. For chemical analyses, florets equivalent to 200–300 g were frozen in liquid N, crushed in a mortar and stored at –80°C until analysis. For sensory analyses, 26 florets were steamed in a steam oven (HBC 26D550702, No100185, Bosh GmbH, München, Germany) until the core temperature of the broccoli floret stems was 90°C, and then steamed for one more minute. The florets were cooled at room temperature for about 3 min, and single frozen in aluminium trays at –20°C. The florets were vacuum packed in boiling-resistant vacuum bags (Goffrato, Scheie & Co, Bergen, Norway) in a single layer and kept in the dark at –20°C until sensory analysis.

Nitrogen and dry matter content of plants. Total N and dry matter (DM) contents of plants were determined by harvesting (cut at soil level) 6–10 broccoli plants at maturity from each plot. The plants were divided into edible parts (broccoli heads) and non-edible parts (leaves and stem). The broccoli fractions were cut in pieces (app. 1–2 cm diameter and length) and mixed. Sub-samples of about 500 g were dried at 60°C for determination of DM and subsequent analysis of total N by the Kjeldahl method [31].

Estimation of potentially plant-available N. Fertilizer-derived N potentially available to plants during the growing season was estimated using data for N mineralization obtained by incubation (unpublished results). Organic materials and waste resources equivalent to 300 kg N ha^{-1} were homogeneously incorporated in soil (50 g DM soil) from the field in Bodø. Soil with and without mixed-in fertilizer material was incubated (Termaks B8420S, Norway, Bergen) at 15 °C for 60 days. Soil moisture was kept at field capacity (–5 kPa) by addition of distilled water twice a week. After 1, 10, 18, 39 and 60 days, triplicates of soil samples from each treatment were sampled and stored at -20 °C. The content of NO_3^--N and NH_4^+-N was determined by extracting 40 g frozen samples in 200 ml 1M KCl prior to analysis. Fertilizer-derived inorganic N was obtained as the difference between fertilized and unfertilized soil. The fertilizer derived N potentially available to plants were determined after an extended phase of only minor changes in measured values. The mean values measured at the last sampling were 53.9%, 54.1% and 86.3% of the N that would correspond to 300 kg ha^{-1} for SM, SS and AD, respectively, while AM immobilized more N than it released (Table 3). The temperature sum at the last sampling during the incubation was 900 degree days, as compared to 823 and 697 in Bodø and 979 and 1116 in Grimstad for the growing seasons of 2009 and 1010, respectively, measured by agricultural climatic services in Norway (LMT), weather stations in Vågønes and Landvik.

Plant N concentration. Total plant N concentration (PNC_{total}) in the above ground part of the broccoli plant (leaf, stem and edible part) was compared to critical plant N concentrations (PNCc) calculated by two different equations: Equation 1 specific for brassica [17] and Equation 2 for arable crops in general [16]:

$$PNCc = 5.2 - 0.178W \qquad \text{(Equation 1)}$$

where W = total DM ha^{-1} < 14.4 t ha^{-1}

$$PNCc = 1.35 + 4.05\, e^{-0.26W} \qquad \text{(Equation 2)}$$

where W = total DM ha^{-1}

Glucosinolate content. For glucosinolate (GLS) analyses, broccoli fertilized with SS, SM and MF corresponding to 170 kg N ha^{-1}, and NF, were chosen. The frozen powder of broccoli florets was freeze-dried (Christ Gamma 1-16, Christ, Osterode, Germany) and ground in a mortar to a fine powder before extraction. Samples for HPLC analysis were prepared according to Vallejo et al. [32] and ISO 9167-1:1992 [33], with several modifications. A sample of about 200 mg of the broccoli powder was placed in a graduated 15 ml tube. The sample tubes were heated at 73 °C in water for 3 min, then 4.5 ml of preheated (73 °C) 70% methanol was added, and the samples were mixed and kept for 3 min at 73 °C. As internal standard, 100 µl of a 2.25 mM glucotropaeolin (Applichem GmbH, Darmstadt, Germany) solution was added. After 10 min at room temperature, the samples were centrifuged at 5300 × *g* for 15 min at 20 °C. The supernatant was decanted into a new tube and the pellet re-extracted with 3.0 ml 70% methanol at room temperature and centrifuged again. The two supernatants were combined, and the extracts were stored at 4 °C until GLS desulphatation the same day. A volume of 0.5 ml DEAE Sephadex suspension (DEAE Sephadex A-25 (GE Healthcare Biosciences AB, Uppsala, Sweden) expanded, washed twice and suspended 1:3 (v/v) in 0.02 M sodium acetate buffer, pH 5.0) was added to a 1 ml syringe fitted with ultrafine glass wool. The column was washed with 0.5 ml water, then 2 × 0.5 ml of sample extract was added and the column was washed again with 2 × 0.5 ml water. The pH was stabilized with 2 × 0.5 ml of 0.2 M sodium acetate buffer (pH 5.0) before 75 µl of purified sulphatase (25 mg ml^{-1} of *Helix pomatia* type H1, Sigma-Aldrich Co., St. Louis, MO, USA) was added. The column was kept at room temperature overnight (at least 11 h). Desulphoglucosinolates were eluted by addition of 0.5 + 0.5 + 0.25 ml of water, and the total eluate was passed through a 0.45 µm Millex®-HV PVDF filter (Merck Millipore Ltd., Cork, Ireland). HPLC analysis was carried out using an Agilent Technologies (Santa Clara, CA, USA) 1100 Series system comprising a quaternary pump, an inline degasser, a thermostat-controlled (5 °C) autosampler, a column heater and a photodiode array detector. Separation was performed on a Spherisorb® ODS2 (Waters Corporation, Milford, MA, USA) 5 µm 4.6 × 250 mm cartridge fitted with a Spherisorb® ODS2 5 µm 4.6 × 10 mm guard column and operated at 30 °C with a flow of 1.5 ml min^{-1}, injection volume of 30 µl and detection at 227 nm. The mobile phases were A: water and B: 20% (v/v) acetonitrile, and the gradient elution program was 1% B for 1 min, linear gradient to 99% B for 20 min, 99% B for 3 min, linear gradient to 1% B for 5 min, then 1% B for 10 min. Desulpho-glucosinolates were identified by comparison of retention times and UV absorbance spectra with those of known standards and on previous mass identification by LC/Q-TOF/MS (Agilent Technologies). Concentrations were calculated from peak areas using response factors relative to glucotropaeolin (ISO 1967-1:1992) and expressed as µmol g^{-1} DM.

Sensory analysis. Prior to sensory analysis, the vacuum-packed broccoli florets were thawed at 4°C overnight. The bags were heated with steam for 6 min at 100°C. The assessors were served broccoli florets of 10–30 g with 2 cm floret stem. Samples were randomized in pairs, and corresponding samples from each locations were analyzed on the same day. The florets were served in preheated porcelain bowls placed on a hot plate. Within each session samples were randomized with respect to serving order. The sensory analyses were carried out during a three-day session.

A descriptive sensory analysis was performed (ISO 6564:1985E) by a trained sensory panel of eight assessors (Nofima, Ås). Twenty-nine sensory attributes within flavor and taste, appearance and color, odor and texture were evaluated. The sensory panel was calibrated using MF and AM fertilized broccoli grown in Grimstad. Appearance and color attributes were evaluated on the larger of the two florets, whereas taste, odor, flavor and texture attributes were evaluated on an average of the two florets. To assess the odor, the assessors cut the florets longitudinally. The texture was evaluated by a bite at the area between the buds and the floret stem, allowing a part of the bud and of the stem to be evaluated. The panelists recorded their results at individual speed on a 15 cm non-structured continuous scale. The data registration system, EyeQuestion, v. 3.8.6 (Logic 8, The Netherlands) transformed the responses from 0 – 15 cm on the screen to numbers from 1.0 (low intensity) to 9.0 (high intensity).

Statistical analysis. Analysis of variance (ANOVA) was performed using general linear model (GLM) in Minitab 16 (Minitab Inc, State College, PA, USA) to determine the statistical effects of design variables on the yield parameters, PNC, GLS and sensory quality parameters. Analysis of variance was also conducted for each location and year for the different treatments. GLM analysis was performed using fertilizer treatment, location, and year as main factors, whereas interactions between main factors and replicates were nested within year and location. For the sensory analyses, individual assessor was considered as random (main) factor, whereas the other factors were fixed. Year and session in sensory analysis were confounded. Tukey's test was used to confirm effect of individual fertilizer treatments.

Regression analysis was performed in Minitab 16 to test the relationship between estimated N from fertilizer materials potentially available to plants during the growing season and measured broccoli yield and GLS content. Pearson correlation analyses were performed to reveal possible relationships between estimated potentially plant-available N, content of total N or total S in fertilizer materials and contents of GLS, and between sensory attributes and phenological expressions (yield, PNC_{total}, fresh weight, N uptake and estimated potentially plant-available N). The correlation analysis was performed for results obtained both years and within each year separately.

Principal component analysis (PCA) was performed using Minitab 16 on yield and N parameters, GLS and statistically significant sensory attributes.

RESULTS

Yield and plant nitrogen concentrations. The yield varied in response to year, location and fertilization (Table 4). The yield ranged from 1.2 Mg ha^{-1} (AM 170 kg N ha^{-1}, Bodø 2010) to 15.4 Mg ha^{-1} (MF 170 kg N ha^{-1}, Grimstad 2010). MF gave significantly higher yield than all other fertilizer treatments except for AD supplied at the rate of 170 kg N ha^{-1}. AM produced yields that were significantly lower compared to the other fertilizer materials at both N rates, and were at similar levels as for NF. There were no significant difference in yield between AD, SS and SM at fertilizer rate of 80 kg N ha^{-1}, but at 170 kg N ha^{-1} AD gave higher yield than SM. Differences were visible as distinct differences in plant size, leaf area and plant height. In Grimstad in 2009, symptoms of N deficiency was observed as broccoli heads tended to be yellowish or violet and poorly developed with high compactness and only single buds reaching maturity. These quality disorders were registered by the sensory panel as degree of uniformity in bud size and color.

The mean PNC_{total} over year and location ranged from 1.7% to 3.0% (Table 4). Significantly higher PNC_{total} was observed in broccoli fertilized with AD and MF, and significantly lower for broccoli fertilized with AM. PNC_c ranged from 4.2% to 4.6% when calculated by Equation 1, and from 2.3% to 3.1% when calculated by Equation 2. The PNC_c calculated by Equation 1 were considerably higher than all PNC_{total}.

The PNC_{total} was higher than PNC_c calculated by Equation 2 in three out the ten fertilizer treatments, and these were AD and SS at a rate of 170 kg ha^{-1} and MF.

Total yield was linearly correlated with estimated amount of inorganic N potentially available from the fertilizer materials during the growing season (Figure 1).

Glucosinolates. The total GLS content was significantly higher for broccoli fertilized with SS and MF (23.0 and 17.1 µmol g^{-1} DM, respectively) (Table 5). These fertilizer materials provide an estimated plant-available N during the growing season corresponding to 92 and 170 kg N ha^{-1} and a high S content of 83 and 81 kg S ha^{-1} for SS and MF, respectively. In contrast, total GLS content in broccoli after SM and NF treatment was significantly lower (11.6 and 13.4 µmol g^{-1} DM, respectively)(Table 5), even though SM corresponds to a plant-available N content of 92 kg ha^{-1} and a S content of 23 kg ha^{-1}. Aliphatic GLS represented 48.3% (SM) to 59.7% (NF) of total GLS content while the indolic GLS represented 39.6% (NF) to 50.4% (SS). Both total aliphatic and total indolic GLS contents were significantly higher in broccoli fertilized with SS compared to SM and NF. Neither total N nor estimated potentially plant-available N derived from fertilizer materials during the growing season correlated with total GLS, total aliphatic or total indolic GLS content. However, when analyzing each year separately correlations between total N or estimated potentially plant-available N and total indolic GLS was found in 2009 (correlation coefficient 0.504 and 0.451 respectively; $p < 0.05$). Correlations were found between S content in added fertilizer materials and total GLS, total aliphatic GLS and total indolic GLS (Correlation coefficient 0.463, 0.362 and 0.495, respectively; $p < 0.05$). Total GLS content was 84.1% higher in 2010 than in 2009**.** Glucoraphanin was the main aliphatic GLS and constituted on average 88.3% of total aliphatic GLS. Glucoraphanin level was significant lower for SM compared to SS and MF, and correlated with S content and N:S ratio in fertilizer (0.389 and -0.320, respectively; $p < 0.05$). Among the individual indolic GLS, differences between fertilizer treatments were observed for glucobrassicin and neoglucobrassicin, which were the main indolic GLSs (on average 43.8 and 46.8%, respectively, of total indolic GLS content). Glucobrassicin was significantly higher for SS and MF, and correlated with total amount of N, estimated potentially plant-available N from fertilizer materials and S content (correlation coefficient 0.378, 0.372 and 0.659, respectively; $p < 0.05$). Significant higher level of neoglucobrassicin was found for SS when compared to NF, and neoglucobrassicin content correlated with S content (correlation coefficient 0.365; $p < 0.05$), and correlated with N content or estimated potentially plant-available N in year 2009 (correlation coefficient 0.483 and 0.436 respectively; $p< 0.05$). Aliphatic GLS level is significant higher in Grimstad than in Bodø.

The ratio between aliphatic and indolic GLS and the ratio between glucoraphanin and glucobrassicin varied with fertilizer treatment and year (Table 5), and were correlated with estimated potentially plant-available N (correlation coefficient -0.338 and -0.468, respectively; $p < 0.05$), total amount of N (correlation coefficient -0.417 and -0.500, respectively; $p < 0.05$) and total S content (correlation coefficient 0.396 and 0.554, respectively; $p < 0.05$) in added fertilizer materials. The ratio between glucoraphanin and neoglucobrassicin was not influenced by fertilizer treatment, but was influenced by year.

Sensory quality. Significant effects of fertilizer materials were observed for 16 out of 29 sensory attributes evaluated (Table 6), however there were no obvious trends in how sensory attributes were influenced. The differences in sensory score for the individual attributes were from 2.2 to 12.2%. In general, sensory attributes were not influenced by location. However, higher levels of sulfur odor and taste were found in Bodø and higher levels of green odor and taste in Grimstad (data not shown).

Sensory attributes were correlated with neither estimated potentially plant-available N nor other phenological expressions such as yield, PNC_{total}, fresh weight and N uptake (data not shown).

Principal component analysis (PCA). The principal component analysis (PCA) of yield, sensory attributes, contents of GLSs and N parameters for fertilization material, location and year shows that 52.0% of the variation could be explained by principal component one and two (Figure 2). In the score plot visualized by fertilizer materials and year, the strongest factor for variable grouping seems to be year. For yield, GLS and N parameters, the year factor is mainly explained by the climate effect. However, for sensory attributes, the climate effect is confounded by possible differences between sensory sessions performed for different years. The score plots show a tendency to grouping by year in two groups. The 2010 samples were located in the right part of the score plot and characterized with high content of GLSs, bitter odor, sour flavor and sour odor. The 2009 samples were located in the left part of score plot and mainly associated with high tendency to uniform bud size, high N content, high score for aftertaste, salty taste, violet color, sulfur flavor and sulfur odor, water flavor and whiteness. Score plot for fertilizer materials show grouping tendency, however there was overlap between source. MF and NF samples were clearly separated in the upper and lower part of the score plot, respectively, with the other fertilizer materials in an intermediate position. MF was associated with high yield, N content, size, fresh weight, and GLS content and high scores for salty taste, aftertaste, violet color, crispness, firmness and sulfur odor. NF samples were associated with sour odor, sour flavor, bitter odor and whiteness as well as high glucoraphanin/ glucobrassicin-ratio and aliphatic/indolic GLS-ratio. Furthermore, broccoli fertilized with SM was associated with high score for uniform bud size and whiteness. Broccoli fertilized with SS was associated with same sensory attributes as MF, but had a stronger association with the different GLS.

DISCUSSION

Yield and plant N concentration. The linear correlation between broccoli yield and estimated potentially plant-available N during the growing season, with no diminishing return, suggest that the optimum N supply was not reached at a rate of 170 kg N ha^{-1}. This is supported by the PNC_{total} being below PNC_c for brassicas (equation 1), indicating that the N availability was sub-optimal even for the fertilizer material with the highest N-supplying potential. However, calculating PNC_c by Equation 2 for arable crops, indicate that broccoli fertilized with SS and AD at 170 kg N ha^{-1} of and MF reached the optimum as PNC_{total} were below PNC_c. The model defining PNC_c for brassica (Equation 1) has previously been found to overestimate the content of N, while PNC_c estimated by Equation 2 for arable crops fits experimental data better or does even underestimate [17, 34]. The N fertilizer rate at 170 kg N ha^{-1} is the upper limit for average N supply rate on arable land in organic farming in Norway. This rate is, however, below the recommended N fertilizer rate for conventional broccoli production in Norway, which is 200–250 kg N ha^{-1} assuming an average marketable yield of 8 – 10 Mg ha^{-1} [35]. Considering the N mineralization from soils and the organic fertilizers' N, the yields in the present study are as expected. This result is in agreement with previous studies showing that N is a growth-limiting nutrient in broccoli production [36-38].

The similarity of recorded yield and PNC_{total} values obtained for broccoli fertilized with SS and AD at the high N rate and those obtained with MF (Table 4) suggests that these fertilizers, when supplied according to the Norwegian regulation for organic agriculture [39], may offer adequate amount and timing of supply of N to meet the demand of broccoli. In contrast, N fertilization with SM and AM was clearly insufficient, which can be explained by different biochemical compositions, notably resulting in higher C to N ratios and, consequently, lower net N mineralization potential (Table 3). In AD, 70% of the N was inorganic and thus potentially plant-available at application time (data not shown). During incubation in soil at 15 °C for 60 days, another 15% of the N was mineralized. On the other hand, for AM there was no net N immobilization during the incubation, which explains the negative fertilizer effect in the present study. This is consistent with the observed linear relationships between potentially plant-available N and yield.

Significant differences found for year and location may be due to climatic conditions. In Bodø, it is likely that the differences in yield between years was influenced by a 1.8 °C lower average temperature and a substantially lower number of sunshine hours in 2010 than in 2009, which may impact both N mineralization in soil as well as broccoli plant growth and development [37, 40, 41]. In addition, large precipitation in 2010, especially around transplanting and during the first weeks of plant development, may have resulted in NO_3^- leaching, and consequently, contributed to the lower N uptake in 2010. In Grimstad, temperature or sunshine hours cannot explain the difference between years, but precipitation may explain the different broccoli size and color

Glucosinolates. The content of GLS was influenced by type of fertilization. The availability of N, S and the N:S ratio has previously been shown to influence the content of GLS [18, 24, 25, 42]. In the present study neither total N supply, estimated potentially plant-available N nor N:S ratio correlated with total GLS content, however there was a positive correlation between total GLS content in broccoli and S content in fertilizer materials. The high total GLS level in broccoli fertilized with SS and MF, which had the highest S content among the fertilizer materials, and the low level of total GLS in broccoli fertilized with SM with low S content, indicate that S supply might be more important for the total GLS content than N supply and N:S ratio at the current fertilizer rates. This is in accordance with previous studies where increasing S supply results in higher total GLS content [43-45]. Li et al. [43] found that increasing N fertilization at high S fertilizer rate did not impact the total GLS content and Vallejo et al. [32] found no differences in total GLS content in broccoli fertilized with increasing N supply (15-150 kg N ha^{-1}). However, the high content of the indolic GLS glucobrassicin in broccoli fertilized with SS and MF compared with SM and NF might be explained by N levels during the growing period as there were correlations between the content of glucobrassicin and both the estimated plant-available N and total N added. These results are in agreement with results obtained for vegetable turnip rape (*Brassica rapa L.*) where the GLS content increased with increasing N regardless of S supply [24]. The higher aliphatic:indolic ratio in broccoli receiving NF is in accordance with previous results, where an increase in indolic GLS and a decrease in aliphatic GLS with increasing N supply have been found [25, 46, 47]. Consequently, the higher content of total GLS content in broccoli fertilized with SS and MF cannot, solely, be explained by variation in the nutritional status for N, but must also be seen in relation to S status and the ratio between N and S.

The high content of GLS in broccoli fertilized with SS might also be due to the content of chitin in shrimp shells. Chitin in SS is the same as chitin found in insect herbivores and may in plants induce stress responses that can influence biosynthesis of GLS, which are phytochemicals important in plant defense [26].

The higher Aliphatic GLS level in Grimstad compered to Bodø is in accordance with Steindal et al [48] results, how found highest aliphatic GLS level in broccoli grown at high temperature in combination with 12 hours daylight.

Sensory attributes. The present study showed only minor effects of fertilizer material and N rate on sensory attributes of broccoli. Some of the differences in sensory attributes may be explained as indirect effects of the applied fertilizers on plant development stage, which have been found to influence sensory attributes [49], rather than direct effects of fertilizer on the sensory properties *per se*. In this study, many broccoli plants fertilized with AM never reached maturity, and the plants appeared very small with high degree of gumminess even at a pre-mature stage. Broccoli fertilized with easily available N matured more evenly, which is in agreement with known effects of N availability on growth and development stage [37, 40, 41]. Differences in sensory attributes of vegetables grown organically and conventionally show inconsistent results [27, 28].

The overall PCA plot showed that year was the most important factor explaining the variation in the samples.

In conclusion, broccoli yield and contents of N and GLS were significantly influenced by type of fertilizer source. Yield increased linearly with estimated potentially plant-available N during the growing season, which resulted in the following yield order: MF>AD>SS>SM>NF>AM. No such linear relationship was found for the GLS content. However, application of SS and MF gave higher contents of some GLS than fertilization with SM and NF. Sensory attributes were more influenced by sensory session (year) than by fertilizer material and location. This study showed that in terms of broccoli crop development and yield, further research on the use of organic and waste-derived fertilizers should focus on determination and prediction of fertilizer-derived plant-available N. When it comes to effects on GLS content, the results suggests a response to the N and S status in fertilizer materials, but more work needs to be done to determine the causes of the measured effects of certain fertilizers. Relatively little is known about effects of climate and other site-specific factors on GLS concentration, which makes it a substantial challenge experimentally to separate fertilizer-specific causal factors from those varying more erratically such as temperature and precipitation.

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Table 1. Planting date (Date), number of growing degree days (GDDs), growth days (GDs) and mean day temperature, total precipitation and total sunshine hours per growing season and month in Bodø and Grimstad for the years 2009 and 2010.

					Mean day temperature (°C)				**Total precipitation (mm)**				**Total sunshine (h)**			
Location	**Year**	**Date**	**GDDs**	**GDs**	Growing season	June	July	August	Growing season	June	July	August	Growing season	June	July	August
Bodø	2009	10th June	823	60	13.7	10.5	14.3	14.4	74.7	51.3	30.5	106.7	507.8	255.7	200.5	141.9
	2010	9th June	697	58	11.9	8.7	13.3	12.4	182.2	91.4	110.3	50.9	274.0	184.8	160.6	152.1
Grimstad	2009	29th May	979	62	15.8	14.9	16.8	15.9	296.4	52.7	243.7	98.6	578.1	276.3	198.7	157.1
	2010	4thJune	1116	68	16.2	15.1	17.0	16.0	198.6	30.1	67.9	130.7	583.8	278.2	199.6	177.4

Table 2. Chemical properties and texture of the upper 0.3 m soil layer of the experimental fields in Bodø and Grimstad 2008.

	Chemical properties						**Texture**		
Location	pH	TC* ($g\ kg^{-1}$)	TN** ($g\ kg^{-1}$)	$N0_3^-$-N ($mg\ kg^{-1}$)	NH_4^+-N ($mg\ kg^{-1}$)	P ($mg\ kg^{-1}$)	Sand	Silt	Clay
Bodø	6.1	21	1.7	7.0	3.9	840	38	52	4
Grimstad	5.9	30	1.6	11.1	1.2	790	87	10	3

*TC=total carbon
**TN=total nitrogen

Table 3. Chemical and physical properties of the organic fertilizers: anaerobically digested food waste (AD), shrimp shell (SS), sheep manure (SM) and algae meal (AM).

													Physical properties
Chemical properties													
Fertilizer	pH	DM %	TOC* (g kg^{-1} DM)	TKN* (g kg^{-1} DM)	NH_4^+-N (g kg^{-1} DM)	NO_3^--N (g kg^{-1} DM)	C:N ratio	EPAN (%)**	P (g kg^{-1} DM)	K (g kg^{-1} DM)	S (g kg^{-1} DM)	N:S ratio	
AD	8.6	1.3	307	254	153	0	1.2	86.3	18	106	8	38.4	liquid part
SS	9.2	90.2	301	72	0	0	4.2	54.1	27	1	4	2.2	dried and pelleted
SM	8.8	19.4	396	37	13	0	17.4	53.9	9	22	5	6.1	solid part, containing traces of straw
AM	6.0	89.1	406	11	0	0	36.9	-24.5	1	16	26	0.4	dried and crushed seaweed, mainly *Ascophyllum nodolus*

*) TOC = Total organic carbon; TKN = Total Kjeldahl Nitrogen
**) EPAN= Estimations of potentially plant-available N based on mineralization from incubation (data not show)

Table 4. Mean values of total yield, quality parameters and nitrogen parameters of broccoli grown with different fertilizers at two locations in Norway (Bodø and Grimstad) in two consecutive years (2009 and 2010).Variables in the same column followed by similar letters are not significantly different by analysis of variance and Tukey's test (p>0.05). Total yield includes broccoli of all sizes.

		Total yield (Mg ha^{-1})	Broccoli head weight (g)	Size-discarded (% of harvested < 6 cm)	Harvested (% of planted)	PNC$_{total}$ % of DM	PNC$_c$, Equation 1[#]	PNC$_c$ Equation 2[##]
*Fertilizer**	*N rate (kg ha^{-1})*							
NF	0	5.9^{de}	170^{de}	5.8^{abc}	84.8^{a}	2.24^{c}	4.55^{a}	3.03^{a}
AM		3.8^{e}	134^{e}	12.8^{a}	66.1^{b}	1.91^{d}	4.53^{ab}	3.05^{a}
AD	80	8.7^{bc}	241^{bc}	5.3^{abc}	88.3^{a}	2.52^{bc}	4.39^{abcde}	2.70^{b}
SS		7.7^{cd}	223^{cd}	2.3^{bc}	85.5^{a}	2.43^{bc}	4.34^{de}	2.61^{bc}
SM		7.1^{cd}	219^{cd}	5.8^{abc}	82.2^{a}	2.35^{bc}	4.43^{abcd}	2.68^{ab}
AM		2.7^{e}	125^{e}	9.9^{ab}	52.6^{c}	1.70^{d}	4.53^{abc}	3.05^{a}
AD		9.8^{ab}	292^{ab}	0.5^{c}	82.4^{a}	2.95^{a}	4.35^{cde}	2.62^{bc}
SS	170	9.1^{bc}	270^{bc}	3.3^{bc}	84.8^{a}	2.67^{ab}	4.25^{ef}	2.48^{bc}
SM		7.8^{c}	234^{c}	2.8^{bc}	82.0^{a}	2.37^{bc}	4.37^{bcde}	2.65^{b}
MF		12.1^{a}	332^{a}	0.4^{c}	88.6^{a}	2.91^{a}	4.16^{f}	2.32^{c}
Year								
2009		8.6	234	6.8	88.6	2.53	4.28	2.46
2010		6.3	214	3.0	70.9	2.28	4.50	3.00
Location								
Grimstad		8.7	266	2.5	83.5	2.14	4.27	2.46
Bodø		6.2	182	7.4	75.9	2.67	4.51	3.00
SEM**		0.33	7.96	0.789	1.63	0.0573	0.0247	0.0535
Treatment		0.000	0.000	0.000	0.000	0.000	0.000	0.000
Year		0.013	0.010	0.003	0.000	0.000	0.000	0.000
Location		0.000	0.000	0.000	0.000	0.000	0.000	0.000
Treatment ×location		NS	NS	NS	NS	NS	NS	NS
Treatment × year		NS	NS	NS	NS	NS	0.001	NS
Year × location		NS	NS	NS	NS	0.000	0.000	0.000
Treatment × year × location		NS	NS	NS	0.014	0.014	NS	NS
Replication (year location)		0.012	0.015	0.001	0.009	NS	NS	NS

*) NF = No fertilizer, AM = Algae meal, AD = Anaerobically digested food waste, SS = Shrimp shell, SM = Sheep manure, MF = Mineral fertilizer

**) SEM = Standard error of the mean, [#]) Greenwood et al, 1996 [##]) Greenwood et al, 1986

Table 5. Mean glucosinolate content (μmol g^{-1} DM) in broccoli grown at two locations (Bodø and Grimstad) and in two years (2009 and 2010) using fertilizers at zero and 170 kg N ha^{-1}.Values followed by the same letters are not significantly different (n=3), Tukey´s test (P<0.05).

Fertilizer *	N rate (kg ha^{-1})	GLS**	ALI	GLI	GLR	IND	4OHGLB	GLB	4MGLB	NGLB	ALI/IND	GLR/GLB	GLR/ NGLB
NF	0	13.36^{b}	8.04^{bc}	1.06^{ab}	9.98^{bc}	5.32^{c}	0.16	2.11^{b}	0.46	2.59^{b}	1.77^{a}	3.65^{a}	3.81
SM	170	10.59^{b}	5.60^{c}	0.68^{b}	4.91^{c}	4.99^{bc}	0.15	1.90^{b}	0.53	2.42^{ab}	1.35^{b}	2.90^{b}	2.77
SS	170	23.00^{a}	11.41^{a}	1.25^{a}	10.16^{a}	11.59^{a}	0.16	5.09^{a}	0.72	5.62^{a}	1.08^{b}	2.03^{b}	2.42
MF	170	17.06^{a}	9.07^{ab}	1.06^{ab}	8.02^{ab}	7.99^{ab}	0.14	3.90^{a}	0.60	3.35^{ab}	1.28^{b}	2.27^{b}	3.03
SEM***		1.240	0.618	0.087	0.534	0.708	0.0232	0.284	0.048	0.413	0.0905	0.177	0.275
Treatment		0.000	0.000	0.012	0.000	0.000	NS	0.000	NS	0.015	0.004	0.000	NS
Year		0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.001	0.014	0.001
Location		NS	0.019	0.003	0.029	NS	0.000	NS	0.001	NS	NS	NS	NS
Treatment × location		NS	0.004	0.021	0.003	NS	NS	NS	NS	NS	NS	NS	NS
Treatment × year		NS	NS	NS	0.110	NS	NS	NS	NS	NS	NS	NS	NS
Location × year		NS	NS	NS	NS	NS	0.001	0.001	NS	NS	0.002	0.000	0.022
Treatment × location × year		NS	NS	NS	NS	NS	NS	0.014	NS	NS	NS	NS	NS
Replication (location year)		0.043	0.008	NS	0.006	NS	NS	NS	NS	NS	NS	NS	NS

* NF = No fertilizer, SM = Sheep manure, SS = Shrimp shell, MF = Mineral fertilizer
** Total glucosinolates, GLS; Total aliphatic, ALI; Total indolic, IND; Glucoiberin, GLI; Glucoraphanin, GLR; 4-Hydroxy-glucobrassicin, 4-OHGLB; Glucobrassicin, GLB; 4-Methoxyglucobrassicin, 4MGLB; Neoglucobrassicin, NGLB.
*** SEM = Standard error of the mean

Table 6. Numeric assessment (from 1 to 9) of selected sensory attributes of broccoli grown with different fertilizers in two years (2009 and 2010) at two locations in Norway (Bodø and Grimstad). Variables in the same column followed by similar letters are not significantly different by analysis of variance and Tukey test (p>0.05).

Fertilizer*	N rate (kg ha^{-1})	Uniform bud size	Whiteness	Violet color	Firmness	Crispness	Juiciness	Astringency	Fibrousness	Sour odor	Bitter odor	Sulfur odor	Sour taste	Salty taste	Sulfur taste	Water taste	After taste
NF	0	6.37^{ab}	3.41^{a}	1.15^{ab}	3.61^{ab}	3.58^{cd}	5.21^{ab}	2.00^{cd}	2.35^{c}	3.58^{a}	3.92^{ab}	3.46^{b}	3.63^{a}	1.46^{bc}	3.64^{ab}	1.97^{ab}	4.59^{cd}
AM	80	5.80^{b}	3.21^{abcd}	1.15^{ab}	3.35^{b}	4.08^{abcd}	5.18^{ab}	2.59^{ab}	2.8^{abc}	2.98^{bc}	3.36^{cde}	3.74^{ab}	2.90^{b}	1.72^{ab}	3.83^{ab}	2.32^{a}	5.21^{ab}
AD		6.02^{ab}	2.88^{d}	1.30^{a}	3.30^{b}	4.07^{abc}	5.07^{ab}	2.63^{a}	2.90^{abc}	2.78^{c}	3.46^{bcde}	3.77^{ab}	2.81^{b}	1.66^{abc}	3.89^{a}	2.25^{ab}	5.20^{ab}
SS		6.09^{ab}	2.94^{bcd}	1.30^{ab}	3.69^{ab}	4.45^{a}	5.42^{a}	2.43^{abc}	2.92^{ab}	3.26^{abc}	3.40^{be}	3.63^{ab}	3.31^{ab}	1.69^{ab}	3.43^{b}	1.88^{ab}	5.03^{abc}
SM		6.04^{ab}	3.26^{abcd}	1.32^{a}	3.53^{b}	4.01^{abcd}	5.08^{ab}	2.64^{a}	3.00^{a}	2.91^{bc}	3.45^{bcde}	3.91^{a}	2.91^{b}	1.71^{a}	3.88^{ab}	2.13^{ab}	5.27^{a}
AM	170	5.90^{ab}	3.52^{a}	1.16^{ab}	3.51^{ab}	3.35^{de}	4.97^{ab}	2.05^{bcd}	2.56^{abc}	3.25^{abc}	3.93^{abcde}	3.74^{b}	3.31^{ab}	1.50^{abc}	3.89^{ab}	2.20^{ab}	4.61^{bcd}
AD		6.28^{ab}	3.35^{abc}	1.14^{ab}	3.41^{b}	3.32^{e}	5.09^{ab}	2.01^{cd}	2.39^{bc}	3.29^{abc}	3.89^{abcde}	3.60^{ab}	3.33^{ab}	1.43^{c}	3.77^{ab}	2.30^{a}	4.60^{cd}
SS		6.49^{a}	3.36^{ab}	1.16^{ab}	3.99^{a}	3.99^{abcd}	5.26^{ab}	1.90^{d}	2.77^{abc}	3.62^{a}	3.83^{abcde}	3.44^{b}	3.66	1.48^{abc}	3.54^{ab}	1.76^{b}	4.53^{d}
SM		6.49^{a}	3.56^{a}	1.11^{b}	3.69^{ab}	3.62^{bcde}	4.96^{b}	2.02^{cd}	2.69^{abc}	3.39^{ab}	3.99^{a}	3.61^{ab}	3.35^{ab}	1.47^{abc}	3.78^{ab}	2.06^{ab}	4.65^{cd}
MF		6.05^{ab}	2.93^{cd}	1.17^{ab}	3.36^{b}	4.16^{ab}	5.29^{ab}	2.41^{abc}	2.68^{abc}	3.09^{abc}	3.41^{e}	3.71^{ab}	3.15^{ab}	1.70^{ab}	3.67^{ab}	2.11^{ab}	5.15^{ab}
SEM**		0.0471	0.0325	0.0142	0.0332	0.0434	0.0318	0.0346	0.0395	0.0410	0.0387	0.0326	0.0417	0.0180	0.0326	0.0372	0.0359
Treatment		0.006	0.000	0.000	0.000	0.000	0.036	0.000	0.002	0.000	0.000	0.038	0.000	0.000	0.023	0.007	0.000
Year /session		0.000	0.000	0.000	0.000	0.000	0.001	0.000	NS	NS	0.000	0.000	0.022	0.001	0.000	0.000	0.000
Location		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.023	NS	NS	0.047	NS	NS
Panellist		0.035	NS	0.001	NS	0.029	0.039	0.000	0.000	0.000	NS	0.054	0.045	NS	0.008	0.000	NS
Treatment × year		0.051	0.006	0.000	NS	0.000	NS	NS	NS	NS	0.001	NS	NS	NS	NS	NS	NS
Treatment × location		NS	NS	0.000	0.010	0.017	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Year × location		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Replication (year location)		NS	NS	0.003	0.000	0.011	NS	NS	0.000	0.005	NS	NS	NS	NS	NS	NS	NS

*NF= No fertilizer, AM= Algae meal, AD= Anaerobically digested food waste, SS= Shrimp shell, SM= Sheep manure, MF= Mineral fertilizer
**SEM = Standard error of the mean

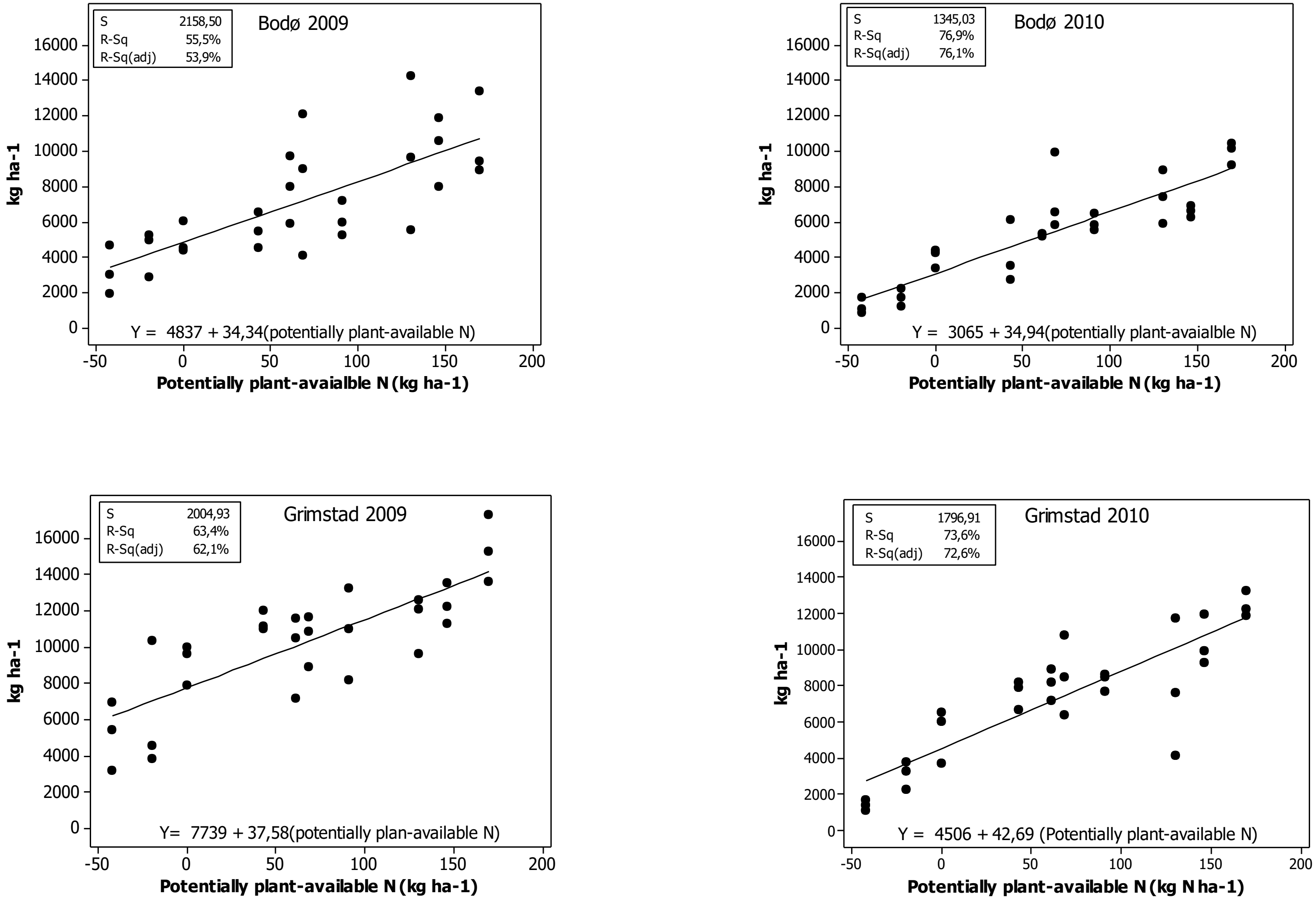

Figure 1. Broccoli yield (kg ha^{-1}) in Bodø and Grimstad 2009 and 2010 regressed on estimated potentially plant-available N (kg ha^{-1}) for the different fertilizers. Estimates are based on mineralization data.

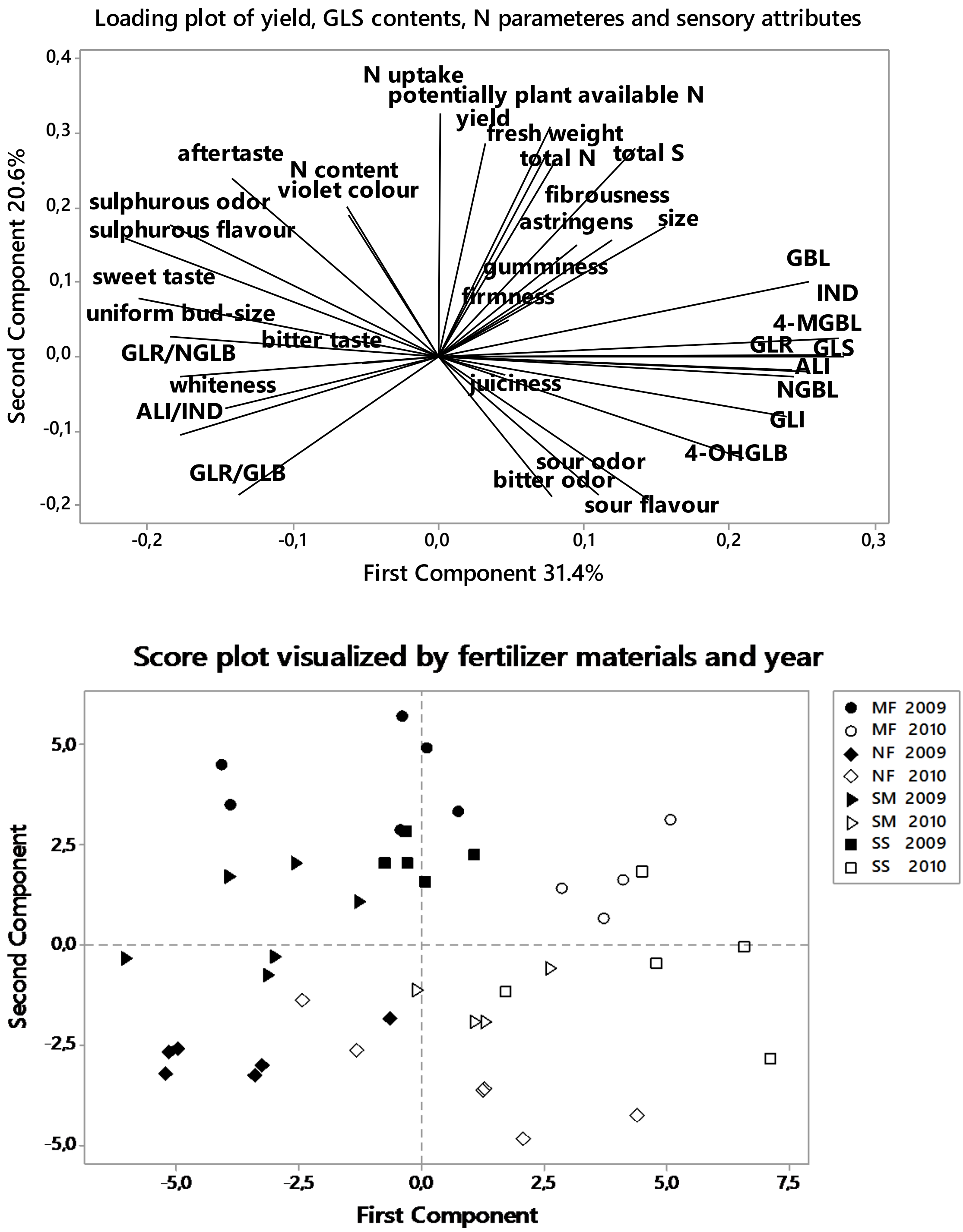

Figure 2. Loading plot and score plots from principal components analysis (PCA) of broccoli grown with different fertilizers materials at a southern (Grimstad) and a northern location (Bodø) for two years (2009 and 2010). The first two principal components explain 52.0% of the variation in GLS content, N and phenological parameters, and sensory attributes. Fertilizer ID abbreviations: NF = No fertilizer, MF= Mineral fertilizer, SM = Sheep manure, SS = Shrimp shell, GLS = Total glucosinolates, ALI = Total aliphatic, IND = Total indolic, GLI = Glucoiberin, GLR = Glucoraphanin, 4-OHGLB = 4-Hydroxy-lucobrassicin, GLB = Glucobrassicin, 4MGLB = 4-Methoxyglucobrassicin, NGLB = Neoglucobrassicin.

For table of content only